includes a number of oligomeric chaperones and some DNA helicases. Here we report a rigorous investigation of the self association properties of the *E. coli* ClpA chaperone, including the ligand linked assembly of the structure active in polypeptide translocation. This has been done by employing sedimentation velocity, sedimentation equilibrium and dynamic light scattering experiments. We also employ rapid mixing kinetic approaches to examine the polypeptide translocation activities of ClpA and ClpAP. Thus far we have shown that ClpA translocates from the carboxy- to amino-terminus without dissociating under single-turnover conditions and thus we consider ClpA to be a processive and directional polypeptide translocase. In addition, we will discuss the first determination of a kinetic step-size for a polypeptide translocase, where the kinetic step-size is defined as the number of amino acids translocated per repeating step.

2109-Pos Board B95
Simulation Studies of the Outer Membrane Protein Chaperone Skp
Peter J. Bond.
Transmembrane outer membrane proteins (OMPs) from Gram-negative bacteria are associated with virulence, multidrug resistance, and general physiological function, and a deeper understanding of OMP biogenesis machinery may provide potential targets for combating diverse pathogens. Skp, a periplasmic chaperone, helps to protect nascent OMPs from degradation, and facilitates their transport to the outer membrane. Skp is a trimeric macromolecule whose structure resembles a "jellyfish", with a small beta-sheet "head" forming the base for long protruding alpha-helical "tentacles" that are thought to sequester a diverse array of unfolded OMP substrates. To further understand the mechanisms of substrate binding by Skp, we have performed all-atom, fully-solvated molecular dynamics simulations, and characterized the inherent conformational flexibility of the trimer via sampling of hundreds of nanoseconds. In addition, Skp has been shown to interact stoichiometrically with bacterial lipopolysaccharide (LPS), a complex glycolipid from the outer membrane of Gram-negative bacteria that regulates release of OMP substrates by unknown mechanisms. Simulations were used to confirm that LPS molecules formed a stable complex with trimeric Skp, bound tightly via their glucosamine-linked phosphate groups to conserved cationic binding sites on the outer surface of the Skp tentacles. Bound LPS induced significant flexibility at glycine-containing hinge regions within the tentacles, leading to opening of the chaperone cavity. This may help to provide a structural basis for the experimentally observed regulation of Skp-bound substrate release by LPS.

2110-Pos Board B96
Beta-Sheet Features in the Greek Key Gamma D-Crystallin Recognized by the Lens Chaperone Alpha B-Crystallin
Ligia Acosta-Sampson, **Jonathan A. King**.
The γ-crystallins are long-lived proteins of the vertebrate lens consisting of four Greek-key motifs organized into two homologous domains. Human γD-Crys can refold in vitro to its native state after unfolding in high concentrations of GdnHCl. However, in buffer or at very low denaturant concentrations aggregation of refolding HγD-Crys intermediates competes with productive refolding. α-crystallin, a small heat shock protein chaperone, is a polydisperse complex of ~ 800 kD consisting of homologous ~20 kD αA- and αB-crystallin chains (αA- and αB-Crys). Its chaperone activity involves suppressing aggregation by binding aggregation-prone species. Aggregates isolated from mature-onset cataracts, the major cause of blindness worldwide, contain damaged and misfolded forms of βγ-crystallins. We had previously determined that the conformation of the bound HγD-Crys substrate in γD_αB complexes resembles the partially folded intermediate populated during refolding/unfolding equilibrium experiments. The chaperone/substrate interaction have been probed further using single domain constructs. The HγD-Crys C-terminal domain construct (γD-Ctd) aggregated upon refolding, while the N-terminal domain construct (γD-Ntd) did not aggregate significantly under similar conditions. HαB-Crys suppressed the aggregation of the γD-Ctd and formed long-lived γD-Ctd_αB complexes. Using a double mutant of HαB-Crys (W9F/W60F) lacking tryptophans, we have determined, through the fluorescence emission of substrate γD-Ctd tryptophans, that the γD-Ctd in the γD-Ctd_αB complexes is partially unfolded. The results are consistent with models in which the tryptophans normally buried in the Greek key, interact with the chaperone in the complex.

2111-Pos Board B97
A Novel ATP-Dependent Conformation in P97 N-D1 Fragment Revealed by Crystal Structures of Disease Related Mutants
Di Xia, Wai Kwan Tang.
Mutations in p97, a major cytosolic AAA chaperone, cause inclusion body myopathy associated with Paget's disease of the bone and frontotemporal dementia (IBMPFD). IBMPFD mutants have single amino acid substitutions at the interface between the N-terminal domain (N-domain) and the adjacent AAA domain (D1), resulting in a reduced affinity for ADP. The structures of p97 N-D1 fragments bearing IBMPFD mutations adopt an atypical N-domain conformation in the presence of Mg2+•ATPγS, which is reversible by ADP, demonstrating for the first time the nucleotide-dependent conformational change of the N-domain. The transition from the ADP- to the ATPγS-bound state is accompanied by a loop-to-helix conversion in the N-D1 linker and by an apparent re-ordering in the N-terminal region of p97. X-ray scattering experiments suggest that wild type p97 subunits undergo a similar nucleotide dependent N-domain conformational change. We propose that IBMPFD mutations alter the timing of the transition between nucleotide states by destabilizing the ADP bound form and consequently interfere with the interactions between the N-domains and their substrates.

2112-Pos Board B98
Insight into the Mechanisms Underlying the Sparing of Masticatory Muscles Function in Acute Quadriplegic Myopathy
Sudhakar Aare.
Acute quadriplegic myopathy (AQM) is a common debilitating acquired disease in intensive care unit (ICU) patients, resulting in tetraplegia/generalized weakness of limb and trunk muscles. Masticatory muscles, on the other hand, are typically spared or less affected than limb/trunk muscles, but the mechanisms underlying the muscle-specific difference remain unknown. Consequently, the aim of this study was to evaluate morphological and physiological parameters together with gene expression profiles of a masticatory muscle, using a unique porcine model mimicking ICU conditions. In this model, animals were immobilized, mechanically ventilated, sedated and exposed to the AQM triggering factors, i.e., neuromuscular blocking agents (NMBA), corticosteroids (CS) and sepsis for five days. Single muscle fiber cross-sectional area and force-generating capacity (specific force), i.e., maximum force normalized to fiber cross-sectional area, and gene expression profiles were analyzed in the masseter muscle samples prior to exposure to triggering factors and after 5 days. Results showed a maintained fiber cross-sectional area and specific force-generating capacity after 5 days exposure to the triggering factors. A total of 825 genes exhibited more than a 2-fold up- or down-regulation. Hence, modifications in heat shock proteins and myostatin genes are in sharp contrast to alterations observed in the limb muscles and it is postulated that elevated heat shock proteins and decreased myostatin genes play a protective role in the masticatory muscle in mechanically ventilated and immobilized ICU patients exposed to NMBA, CS and sepsis.

2113-Pos Board B99
Gly Scan of Prouroguanylin to Investigate Essential Amino Acid Residues for the Intra-Molecular Chaperone Function
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Peptide hormones are often produced in the form of the precursor proteins with prepro-sequences *in vivo*. The pre-region acts as a signal peptide to carry the precursor protein to endoplasmic reticulum. However, little is known concerning the role of pro-sequences in peptide hormones.
Uroguanylin, an endogenous ligand of guanylyl cyclase C, is also produced via the processing of the precursor protein, prouroguanylin. We previously reported that the pro-peptide region in prouroguanylin functions as an intramolecular chaperone in the correct folding of the mature peptide, uroguanylin. Furthermore, folding analyses of the N-terminal mutants of prouroguanylin suggested that the Ile^3 residue in prouroguanylin plays an important role in the chaperone function of pro-peptide region.
Recently, the NMR structural analysis of proguanylin, related protein to prouroguanylin, suggested that the N-terminal region of pro-peptide interacts with the mature region to stabilize the tertiary structure of the mature peptide. However, the role of each amino acid residue in pro-peptide region remains to be studied. Therefore, we further performed the sited-directed mutagenesis for prouroguanylin to estimate the role of each amino acid residue in the intramolecular chaperone function.
For this purpose, Gly scanning was employed to estimate the role of side chains of each amino acid residue for the intra-molecular chaperone function. Each N-terminal amino acid residue of the pro-peptide region was sequentially mutated to a Gly residue. Mutants were prepared using the T_7-promoter expression system in *E. coli* cells. After the purification of the reduced form of mutants by RP HPLC, the refolding reactions were carried out in the presence of glutathione. The folding reaction was monitored by RP-HPLC and the Circular Dichroism measurement. Folding products were analyzed by MALDI-TOF/MS. The results will be discussed in this paper.